

Hepatoprotective effects of systemic ER activation

ChIPseq/Epigenome genome - Differential binding analysis

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```
library(tidyverse)
library(DiffBind)
```

```
sessionInfo()
```

```
## R version 4.2.3 (2023-03-15)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur ... 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRlapack.dylib
##
## locale:
##  [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats4      stats      graphics  grDevices  utils      datasets  methods
## [8] base
##
## other attached packages:
##  [1] DiffBind_3.8.4           SummarizedExperiment_1.28.0
##  [3] Biobase_2.58.0           MatrixGenerics_1.10.0
##  [5] matrixStats_1.0.0       GenomicRanges_1.50.2
##  [7] GenomeInfoDb_1.34.9     IRanges_2.32.0
##  [9] S4Vectors_0.36.2       BiocGenerics_0.44.0
## [11] lubridate_1.9.2         forcats_1.0.0
## [13] stringr_1.5.0          dplyr_1.1.2
## [15] purrr_1.0.2            readr_2.1.4
## [17] tidyr_1.3.0            tibble_3.2.1
## [19] ggplot2_3.4.3          tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
##  [1] bitops_1.0-7           RColorBrewer_1.1-3     numDeriv_2016.8-1.1
##  [4] tools_4.2.3           irlba_2.3.5.1         utf8_1.2.3
##  [7] R6_2.5.1              KernSmooth_2.23-20    DBI_1.1.3
## [10] colorspace_2.1-0      apeglm_1.20.0         withr_2.5.0
## [13] tidyselect_1.2.0      compiler_4.2.3        cli_3.6.1
## [16] DelayedArray_0.24.0   rtracklayer_1.58.0    caTools_1.18.2
```

```
## [19] scales_1.2.1          SQUAREM_2021.1      mvtnorm_1.2-2
## [22] mixsqp_0.3-48         digest_0.6.33       Rsamtools_2.14.0
## [25] rmarkdown_2.23        XVector_0.38.0      jpeg_0.1-10
## [28] pkgconfig_2.0.3       htmltools_0.5.5     BSgenome_1.66.3
## [31] invgamma_1.1          fastmap_1.1.1       bbmle_1.0.25
## [34] limma_3.54.2          htmlwidgets_1.6.2   rlang_1.1.1
## [37] rstudioapi_0.15.0     BiocIO_1.8.0        generics_0.1.3
## [40] hwriter_1.3.2.1       BiocParallel_1.32.6 gtools_3.9.4
## [43] RCurl_1.98-1.12       magrittr_2.0.3      GenomeInfoDbData_1.2.9
## [46] interp_1.1-4          Matrix_1.5-3        Rcpp_1.0.11
## [49] munsell_0.5.0         fansi_1.0.4         lifecycle_1.0.3
## [52] stringi_1.7.12        yaml_2.3.7          MASS_7.3-58.2
## [55] zlibbioc_1.44.0       plyr_1.8.8          gplots_3.1.3
## [58] grid_4.2.3           parallel_4.2.3      ggrepel_0.9.3
## [61] bdsmatrix_1.3-6       crayon_1.5.2        deldir_1.0-9
## [64] lattice_0.20-45       Biostrings_2.66.0   hms_1.1.3
## [67] locfit_1.5-9.8        knitr_1.43          pillar_1.9.0
## [70] rjson_0.2.21          systemPipeR_2.4.0   codetools_0.2-19
## [73] XML_3.99-0.14         glue_1.6.2          evaluate_0.21
## [76] ShortRead_1.56.1      GreyListChIP_1.30.0 latticeExtra_0.6-30
## [79] png_0.1-8            vctrs_0.6.3         tzdb_0.4.0
## [82] gtable_0.3.3          amap_0.8-19         ashR_2.2-54
## [85] emdbook_1.3.13        xfun_0.39           restfulr_0.0.15
## [88] coda_0.19-4          truncnorm_1.0-9     GenomicAlignments_1.34.1
## [91] timechange_0.2.0
```

```
getwd()
```

```
## [1] "/Users/christiansom/Documents/GitHub/MAFLD_ER_agonists"
```

```
samples <- c("CD2", "CD6", "CD9", "HFD3", "HFD4", "HFD6", "DPN2", "DPN3", "DPN6", "DIP3", "DIP6", "DIP10", "E2", "E8", "E9", "PPT1", "PPT2", "PPT3")
Factor_K27 <- c(rep("H3K27ac", 18))
PeakCaller <- c(rep("macs", 18))
Replicate <- c(rep(1:3, 6))
Treatment <- c(rep("CD", 3), rep("HFD", 3), rep("DPN", 3), rep("DIP", 3), rep("E2", 3), rep("PPT", 3))
files_K27 <- c("data/files/Peak_calling_MACS2/231128_CK0744_H3K27ac_CD2_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_CD6_H3K27ac_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_CK0745_H3K27ac_CD9_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_CK0746_H3K27ac_HFD3_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_CK0747_H3K27ac_HFD4_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_HFD6_H3K27ac_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_CK0748_H3K27ac_DPN2_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_CK0749_H3K27ac_DPN3_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_DPN6_H3K27ac_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_DIP3_H3K27ac_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_DIP6_H3K27ac_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_DIP10_H3K27ac_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_E2_2_H3K27ac_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_CK0750_H3K27ac_E2_8_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_CK0751_H3K27ac_E2_9_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_PPT1_H3K27ac_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_PPT2_H3K27ac_peaks.broadPeak",
              "data/files/Peak_calling_MACS2/231128_PPT3_H3K27ac_peaks.broadPeak")
```

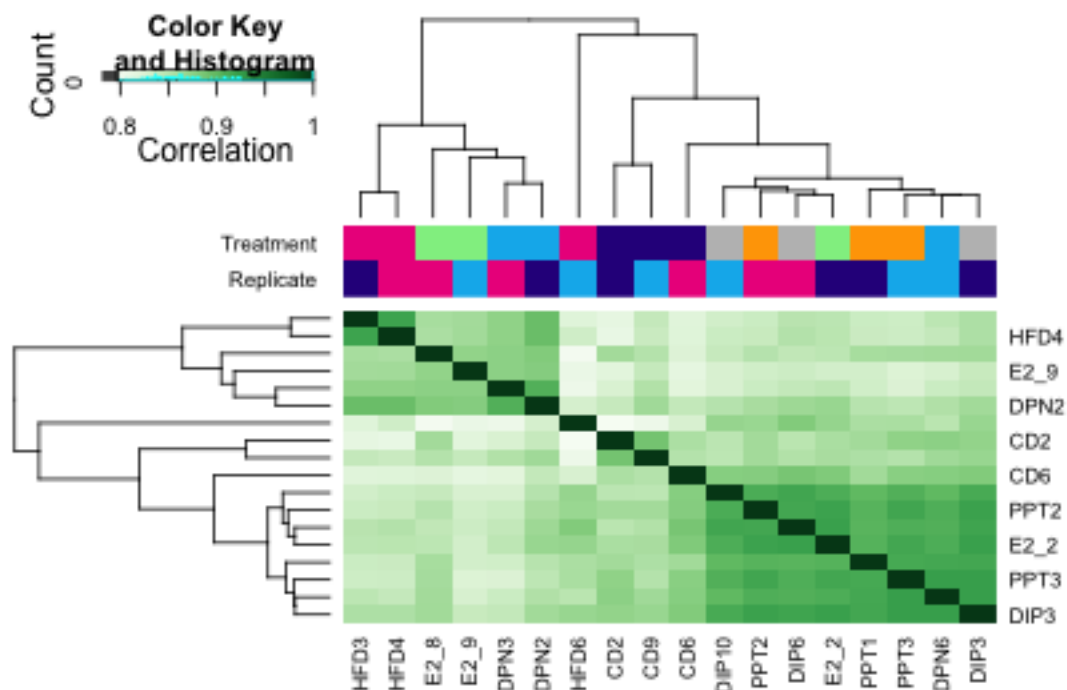
```

bam.files_K27 <- c("data/files/BAMdata/H3K27ac/231127_CK0744_H3K27ac_CD2_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_CD6_H3K27ac_S2_R1_001_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_CK0745_H3K27ac_CD9_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_CK0746_H3K27ac_HFD3_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_CK0747_H3K27ac_HFD4_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_HFD6_H3K27ac_S8_R1_001_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_CK0748_H3K27ac_DPN2_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_CK0749_H3K27ac_DPN3_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_DPN6_H3K27ac_S15_R1_001_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_DIP3_H3K27ac_S9_R1_001_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_DIP6_H3K27ac_S10_R1_001_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_DIP10_H3K27ac_S11_R1_001_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_E2_2_H3K27ac_S16_R1_001_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_CK0750_H3K27ac_E2_8_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_CK0751_H3K27ac_E2_9_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_PPT1_H3K27ac_S12_R1_001_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_PPT2_H3K27ac_S13_R1_001_psort_BL_fix_MkDup.bam",
  "data/files/BAMdata/H3K27ac/231127_PPT3_H3K27ac_S14_R1_001_psort_BL_fix_MkDup.bam")

metaData_K27 <- data.frame(SampleID = samples, Factor = Factor_K27, Replicate = Replicate, Peaks = files)
samplesheet_K27 <- dba(sampleSheet=metaData_K27)

```

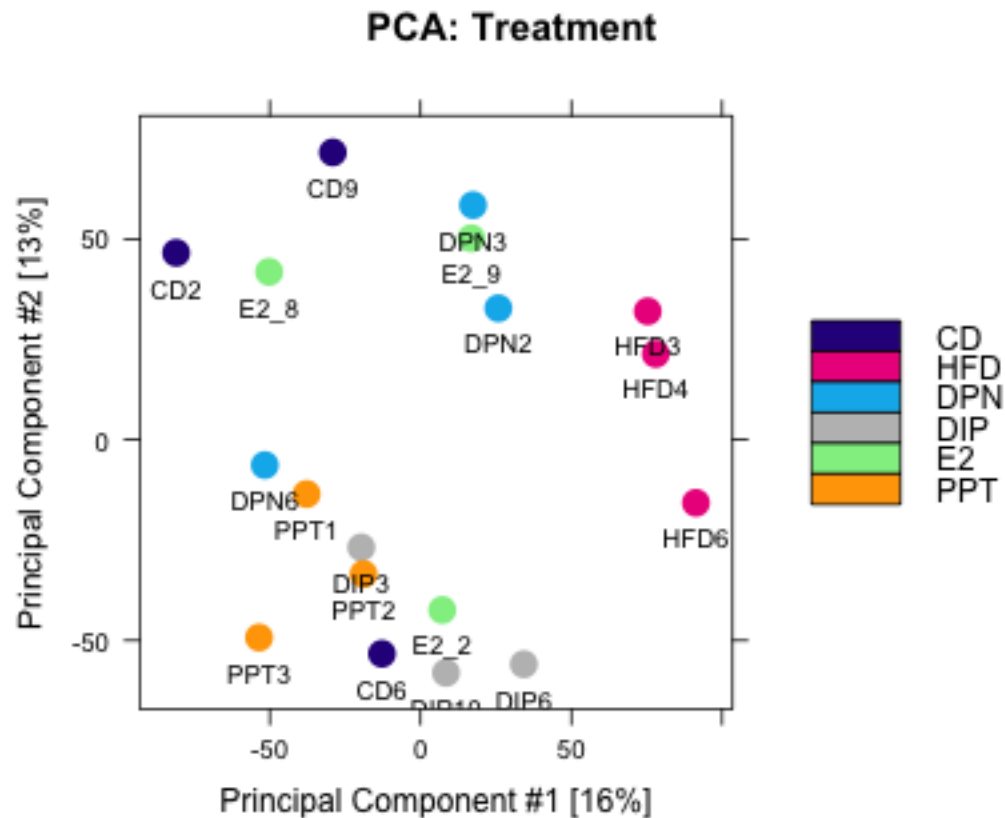
```
dba.plotHeatmap(samplesheet_K27)
```



```
samplesheet.counted_K27 <- dba.count(samplesheet_K27, minOverlap = 2)
dba.overlap(samplesheet_K27, mode=DBA_OLAP_RATE)
```

```
## [1] 77016 59463 52812 48728 45735 43432 41480 39805 38306 36957 35662 34403
## [13] 33159 31930 30615 29154 27484 25079
```

```
#That is a PCA based on read counts
dba.plotPCA(samplesheet.counted_K27, label=DBA_ID)
```



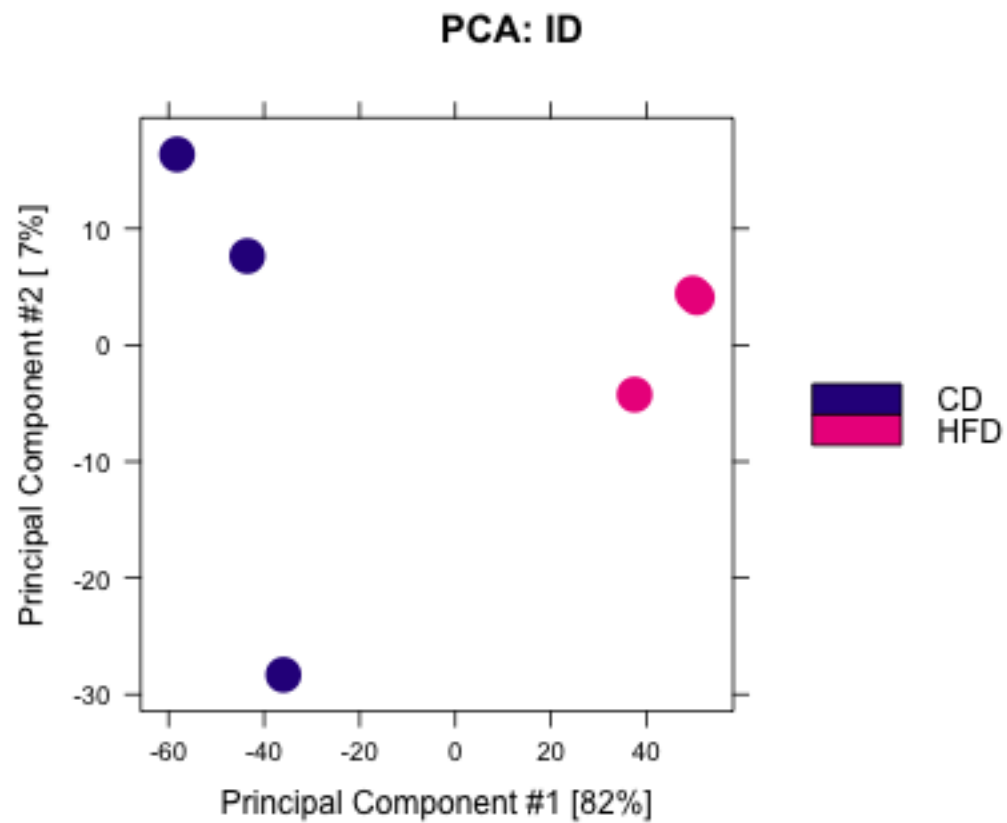
```
contrasts_K27 <- dba.contrast(samplesheet.counted_K27, categories=c(DBA_TREATMENT), minMembers=2)
samplesheet.analysed_K27 <- dba.analyze(contrasts_K27)
```

```
#That is a PCA based on diffbound sites of given contrast
##Get contrast numbers
dba.show(samplesheet.analysed_K27, bContrast=T)
```

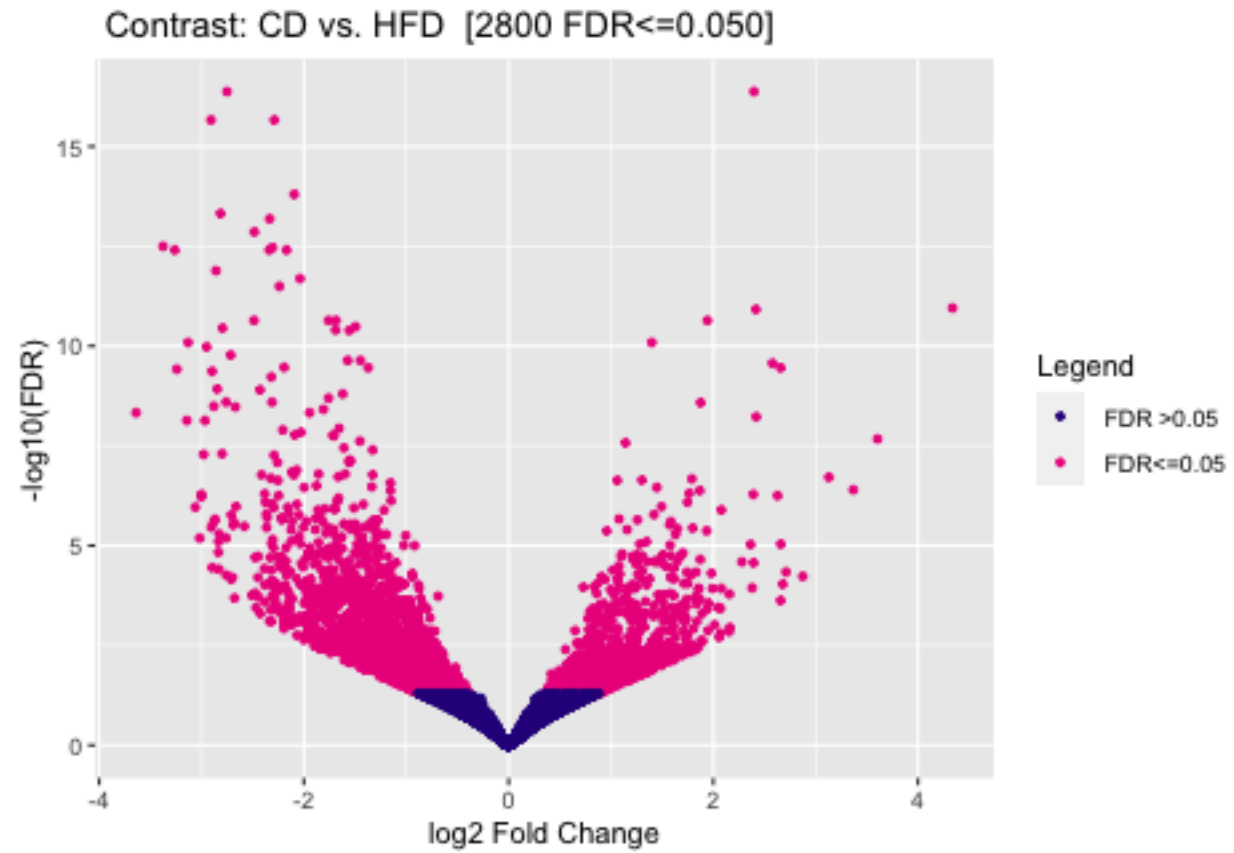
```
##      Factor Group Samples Group2 Samples2 DB.DESeq2
## 1 Treatment   CD       3      HFD         3      2800
## 2 Treatment   CD       3      DPN         3       104
## 3 Treatment   CD       3      DIP         3       227
## 4 Treatment   CD       3      E2          3       166
## 5 Treatment   CD       3      PPT         3       136
## 6 Treatment  HFD       3      DPN         3       562
```

## 7	Treatment	HFD	3	DIP	3	77
## 8	Treatment	HFD	3	E2	3	644
## 9	Treatment	HFD	3	PPT	3	997
## 10	Treatment	DPN	3	DIP	3	2
## 11	Treatment	DPN	3	E2	3	13
## 12	Treatment	DPN	3	PPT	3	5
## 13	Treatment	DIP	3	E2	3	1
## 14	Treatment	DIP	3	PPT	3	2
## 15	Treatment	PPT	3	E2	3	0

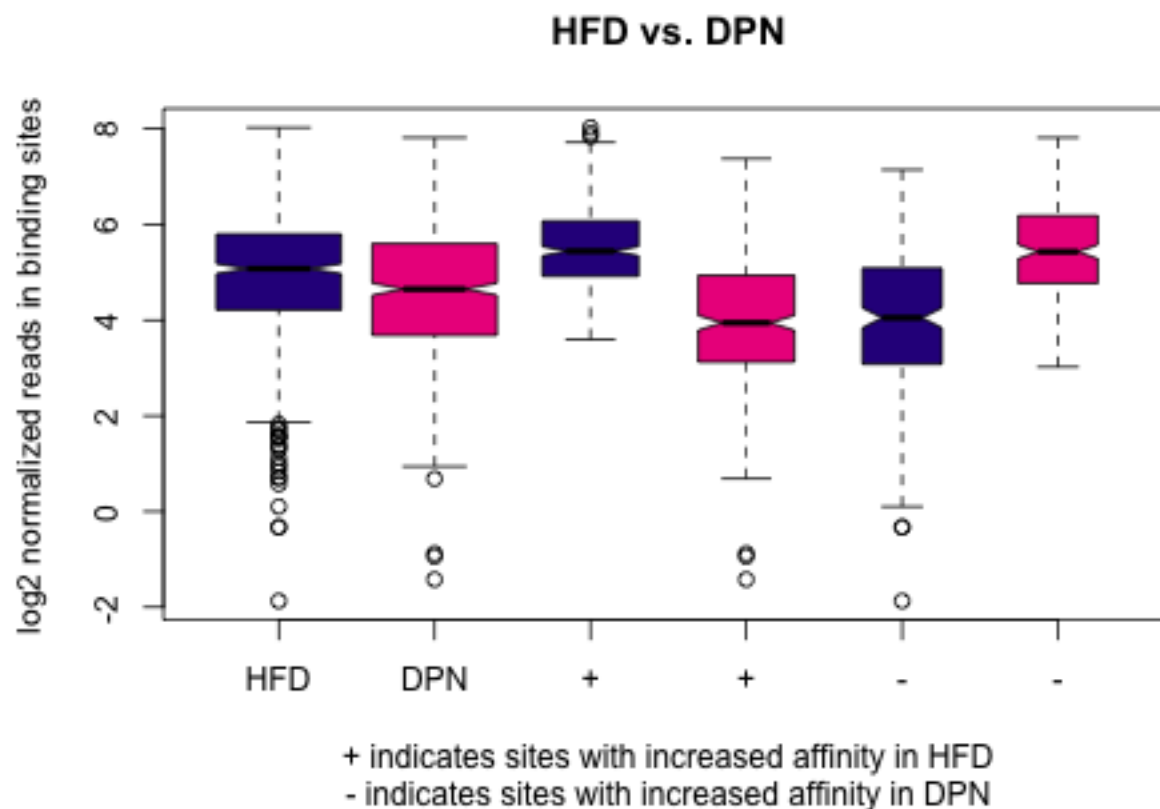
```
dba.plotPCA(samplesheet.analysed_K27, contrast = 1)
```



```
dba.plotVolcano(samplesheet.analysed_K27, contrast = 1)
```



```
dba.plotBox(samplesheet.analysed_K27, contrast =6)
```



Choose appropriate contrasts, export the full tables, then filter for significant changes with a minimum foldchange of 50% up or down. # Then split the peaks into Loss/Gain in HFD and export bed files with these sites.

`samplesheet.analysed_K27`

```
## 18 Samples, 57716 sites in matrix:
##      ID Factor Treatment Replicate   Reads FRiP
## 1   CD2 H3K27ac      CD           1 21227665 0.17
## 2   CD6 H3K27ac      CD           2 12314978 0.20
## 3   CD9 H3K27ac      CD           3 18625961 0.16
## 4   HFD3 H3K27ac     HFD           1 19237373 0.17
## 5   HFD4 H3K27ac     HFD           2 20167826 0.17
## 6   HFD6 H3K27ac     HFD           3 12831261 0.18
## 7   DPN2 H3K27ac     DPN           1 19492675 0.16
## 8   DPN3 H3K27ac     DPN           2 21083300 0.16
## 9   DPN6 H3K27ac     DPN           3 14175773 0.19
## 10  DIP3 H3K27ac     DIP           1 13121224 0.20
## 11  DIP6 H3K27ac     DIP           2 13362305 0.20
## 12  DIP10 H3K27ac    DIP           3 11599539 0.19
## 13  E2_2 H3K27ac     E2            1 14096089 0.20
## 14  E2_8 H3K27ac     E2            2 18028631 0.17
## 15  E2_9 H3K27ac     E2            3 18884119 0.16
## 16  PPT1 H3K27ac     PPT            1 11979475 0.19
## 17  PPT2 H3K27ac     PPT            2 12519231 0.18
## 18  PPT3 H3K27ac     PPT            3 12723121 0.19
##
```

```
## Design: [~Treatment] | 15 Contrasts:
```

##	Factor	Group	Samples	Group2	Samples2	DB.DESeq2
## 1	Treatment	CD	3	HFD	3	2800
## 2	Treatment	CD	3	DPN	3	104
## 3	Treatment	CD	3	DIP	3	227
## 4	Treatment	CD	3	E2	3	166
## 5	Treatment	CD	3	PPT	3	136
## 6	Treatment	HFD	3	DPN	3	562
## 7	Treatment	HFD	3	DIP	3	77
## 8	Treatment	HFD	3	E2	3	644
## 9	Treatment	HFD	3	PPT	3	997
## 10	Treatment	DPN	3	DIP	3	2
## 11	Treatment	DPN	3	E2	3	13
## 12	Treatment	DPN	3	PPT	3	5
## 13	Treatment	DIP	3	E2	3	1
## 14	Treatment	DIP	3	PPT	3	2
## 15	Treatment	PPT	3	E2	3	0

```
res_deseq_K27_CD_vs_HFD <- as.data.frame(dba.report(samplesheet.analysed_K27, method=DBA_DESEQ2, contrast=CDvsHFD))
res_deseq_K27_HFD_vs_DPN <- as.data.frame(dba.report(samplesheet.analysed_K27, method=DBA_DESEQ2, contrast=HFDvsDPN))
res_deseq_K27_HFD_vs_DIP <- as.data.frame(dba.report(samplesheet.analysed_K27, method=DBA_DESEQ2, contrast=HFDvsDIP))
res_deseq_K27_HFD_vs_E2 <- as.data.frame(dba.report(samplesheet.analysed_K27, method=DBA_DESEQ2, contrast=HFDvsE2))
res_deseq_K27_HFD_vs_PPT <- as.data.frame(dba.report(samplesheet.analysed_K27, method=DBA_DESEQ2, contrast=HFDvsPPT))
res_deseq_K27_DPN_vs_DIP <- as.data.frame(dba.report(samplesheet.analysed_K27, method=DBA_DESEQ2, contrast=DPNvsDIP))
res_deseq_K27_DPN_vs_E2 <- as.data.frame(dba.report(samplesheet.analysed_K27, method=DBA_DESEQ2, contrast=DPNvsE2))
res_deseq_K27_DPN_vs_PPT <- as.data.frame(dba.report(samplesheet.analysed_K27, method=DBA_DESEQ2, contrast=DPNvsPPT))
```

```
# Just too see how many different peaks are identified amongst the treatments. We wont need these peaks
nrow(res_deseq_K27_DPN_vs_DIP %>% dplyr::filter(FDR< 0.05 & abs(Fold) > 0.585))
```

```
## [1] 2
```

```
nrow(res_deseq_K27_DPN_vs_E2 %>% dplyr::filter(FDR< 0.05 & abs(Fold) > 0.585))
```

```
## [1] 13
```

```
nrow(res_deseq_K27_DPN_vs_PPT %>% dplyr::filter(FDR< 0.05 & abs(Fold) > 0.585))
```

```
## [1] 4
```

```
Diffbind_tables <- list()
Diffbind_tables$unfiltered <- list(K27_CDvsHFD = res_deseq_K27_CD_vs_HFD,
                                   K27_HFDvsDPN = res_deseq_K27_HFD_vs_DPN,
                                   K27_HFDvsE2 = res_deseq_K27_HFD_vs_E2,
                                   K27_HFDvsPPT = res_deseq_K27_HFD_vs_PPT)
```

```
Diffbind_tables$all_DB_peaks <- list()
Diffbind_tables$up_DB_peaks <- list()
Diffbind_tables$down_DB_peaks <- list()
```

```
Diffbind_bed_files <- list()
```



```

all_DB_peaks=list(),
up_DB_peaks=list(),
down_DB_peaks=list())
# This loop saves the diffbound peaks in the diffbind_tables list
# This loop also saves the bedfiles in a separate list

for (i in 1:length(Diffbind_tables$unfiltered)) {

  ### PART 1 - Put the up- and downregulated peak objects (in the given contrast orientations) into dataframes
  #Both up-and downregulated together
  Diffbind_tables$all_DB_peaks[[i]] <- Diffbind_tables$unfiltered[[i]] %>% dplyr::filter(FDR< 0.05 & abs(Fold) > 0.585)
  #Only the upregulated peaks
  Diffbind_tables$up_DB_peaks[[i]] <- Diffbind_tables$unfiltered[[i]] %>% dplyr::filter(FDR< 0.05 & Fold > 0.585)
  #Only the downregulated peaks
  Diffbind_tables$down_DB_peaks[[i]] <- Diffbind_tables$unfiltered[[i]] %>% dplyr::filter(FDR< 0.05 & Fold < -0.585)

  ### PART 2 - Convert the objects to bedgraph format.
  Diffbind_bed_files$all_DB_peaks[[i]] <- Diffbind_tables$unfiltered[[i]] %>%
    dplyr::filter(FDR< 0.05 & abs(Fold) > 0.585) %>%
    dplyr::select(1:3) %>% mutate(name = "NA") %>% mutate(strand = "NA")

  Diffbind_bed_files$up_DB_peaks[[i]] <- Diffbind_tables$unfiltered[[i]] %>%
    dplyr::filter(FDR< 0.05 & Fold > 0.585) %>%
    dplyr::select(1:3) %>% mutate(name = "NA") %>% mutate(strand = "NA")

  Diffbind_bed_files$down_DB_peaks[[i]] <- Diffbind_tables$unfiltered[[i]] %>%
    dplyr::filter(FDR< 0.05 & Fold < -0.585) %>%
    dplyr::select(1:3) %>% mutate(name = "NA") %>% mutate(strand = "NA")
}

names(Diffbind_tables$all_DB_peaks) <- names(Diffbind_tables$unfiltered)
names(Diffbind_tables$up_DB_peaks) <- names(Diffbind_tables$unfiltered)
names(Diffbind_tables$down_DB_peaks) <- names(Diffbind_tables$unfiltered)
names(Diffbind_bed_files$all_DB_peaks) <- names(Diffbind_tables$unfiltered)
names(Diffbind_bed_files$up_DB_peaks) <- names(Diffbind_tables$unfiltered)
names(Diffbind_bed_files$down_DB_peaks) <- names(Diffbind_tables$unfiltered)

saveRDS(Diffbind_tables, "results/Epigenome_analysis/Diffbind_results_FDR_fold.rds")

# Export the bedfiles. These will be used later in the analysis.

for (i in 1:length(Diffbind_bed_files)) {

  write.table(x = Diffbind_bed_files$all_DB_peaks[[i]], file=paste0("results/Epigenome_analysis/Diffbind_results_FDR_fold_",
    names(Diffbind_bed_files$all_DB_peaks[i]), ".bed"), sep="\t", quote=F, as.is=T)

  write.table(x = Diffbind_bed_files$up_DB_peaks[[i]], file=paste0("results/Epigenome_analysis/Diffbind_results_FDR_fold_",
    names(Diffbind_bed_files$up_DB_peaks[i]), ".bed"), sep="\t", quote=F, as.is=T)

  write.table(x = Diffbind_bed_files$down_DB_peaks[[i]], file=paste0("results/Epigenome_analysis/Diffbind_results_FDR_fold_",
    names(Diffbind_bed_files$down_DB_peaks[i]), ".bed"), sep="\t", quote=F, as.is=T)
}

```

```
#load the packages for the annotations
```

```
library("ChIPpeakAnno")
library("GenomicRanges")
options(connectionObserver = NULL) #That is a work-around, as the org.Mm. package cannot be loaded
library("org.Mm.eg.db")
library("biomaRt")
```

```
#Annotate all of the coordinates of DiffBound peaks in the different objects. Nearest gene.
```

```
# For annotation, we use the September 2019 version, which was also used for RNAseq.
listEnsemblArchives()
```

```
##           name      date                url version
## 1  Ensembl GRCh37 Feb 2014      https://grch37.ensembl.org GRCh37
## 2    Ensembl 110 Jul 2023 https://jul2023.archive.ensembl.org    110
## 3    Ensembl 109 Feb 2023 https://feb2023.archive.ensembl.org    109
## 4    Ensembl 108 Oct 2022 https://oct2022.archive.ensembl.org    108
## 5    Ensembl 107 Jul 2022 https://jul2022.archive.ensembl.org    107
## 6    Ensembl 106 Apr 2022 https://apr2022.archive.ensembl.org    106
## 7    Ensembl 105 Dec 2021 https://dec2021.archive.ensembl.org    105
## 8    Ensembl 104 May 2021 https://may2021.archive.ensembl.org    104
## 9    Ensembl 103 Feb 2021 https://feb2021.archive.ensembl.org    103
## 10   Ensembl 102 Nov 2020 https://nov2020.archive.ensembl.org    102
## 11   Ensembl 101 Aug 2020 https://aug2020.archive.ensembl.org    101
## 12   Ensembl 100 Apr 2020 https://apr2020.archive.ensembl.org    100
## 13   Ensembl 99 Jan 2020 https://jan2020.archive.ensembl.org     99
## 14   Ensembl 98 Sep 2019 https://sep2019.archive.ensembl.org     98
## 15   Ensembl 97 Jul 2019 https://jul2019.archive.ensembl.org     97
## 16   Ensembl 96 Apr 2019 https://apr2019.archive.ensembl.org     96
## 17   Ensembl 95 Jan 2019 https://jan2019.archive.ensembl.org     95
## 18   Ensembl 94 Oct 2018 https://oct2018.archive.ensembl.org     94
## 19   Ensembl 93 Jul 2018 https://jul2018.archive.ensembl.org     93
## 20   Ensembl 80 May 2015 https://may2015.archive.ensembl.org     80
## 21   Ensembl 77 Oct 2014 https://oct2014.archive.ensembl.org     77
## 22   Ensembl 75 Feb 2014 https://feb2014.archive.ensembl.org     75
## 23   Ensembl 54 May 2009 https://may2009.archive.ensembl.org     54
##      current_release
## 1
## 2          *
## 3
## 4
## 5
## 6
## 7
## 8
## 9
## 10
## 11
## 12
## 13
## 14
## 15
```

```
## 16
## 17
## 18
## 19
## 20
## 21
## 22
## 23
```

```
mart <- useMart(biomart = "ensembl", dataset = "mmusculus_gene_ensembl", host = "https://sep2019.archive.
# use the getAnnotation function to obtain the TSS for ensembl GRCh38.p13.
annoData <- getAnnotation(mart, featureType = "TSS")

# Annotate the peak files.
Diffbind_tables$annotated <- Diffbind_tables

for (i in 1:length(Diffbind_tables$annotated)) {
  for (k in 1:length(Diffbind_tables$annotated$unfiltered)) {

    colnames(Diffbind_tables$annotated[[i]][[k]]) <- c("chrom", "start", "end")
    nrow(Diffbind_tables$annotated[[i]][[k]])

    Diffbind_tables$annotated[[i]][[k]] <- makeGRangesFromDataFrame(Diffbind_tables$annotated[[i]][[k]],
                                                                    start.field = "start", end.field = "end")

    #Give ranges numeric names in order
    names(Diffbind_tables$annotated[[i]][[k]]) <- c(1:length(Diffbind_tables$annotated[[i]][[k]]))

    #Annotate granges with the nearest TSS
    Diffbind_tables$annotated[[i]][[k]] <- annotatePeakInBatch(Diffbind_tables$annotated[[i]][[k]],
                                                              AnnotationData=annoData,
                                                              featureType = "TSS",
                                                              output="nearestLocation",
                                                              PeakLocForDistance = "start")

    Diffbind_tables$annotated[[i]][[k]] <- as.data.frame(Diffbind_tables$annotated[[i]][[k]]) %>% remove_row
  }
}

saveRDS(Diffbind_tables$annotated, file="results/Epigenome_analysis/Diffbind_all_annotated_DB_peaks.rds")
```

```
sessionInfo()
```

```
## R version 4.2.3 (2023-03-15)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur ... 10.16
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRlapack.dylib
##
## locale:
```

```

## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats4      stats      graphics  grDevices  utils      datasets  methods
## [8] base
##
## other attached packages:
## [1] biomaRt_2.54.1          org.Mm.eg.db_3.16.0
## [3] AnnotationDbi_1.60.2    ChIPpeakAnno_3.32.0
## [5] DiffBind_3.8.4          SummarizedExperiment_1.28.0
## [7] Biobase_2.58.0          MatrixGenerics_1.10.0
## [9] matrixStats_1.0.0       GenomicRanges_1.50.2
## [11] GenomeInfoDb_1.34.9     IRanges_2.32.0
## [13] S4Vectors_0.36.2        BiocGenerics_0.44.0
## [15] lubridate_1.9.2         forcats_1.0.0
## [17] stringr_1.5.0           dplyr_1.1.2
## [19] purrr_1.0.2             readr_2.1.4
## [21] tidyr_1.3.0             tibble_3.2.1
## [23] ggplot2_3.4.3           tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
## [1] BiocFileCache_2.6.1      plyr_1.8.8              lazyeval_0.2.2
## [4] splines_4.2.3           BiocParallel_1.32.6     amap_0.8-19
## [7] digest_0.6.33           ensemblDb_2.22.0        invgamma_1.1
## [10] htmltools_0.5.5         SQUAREM_2021.1          fansi_1.0.4
## [13] magrittr_2.0.3          memoise_2.0.1           BSgenome_1.66.3
## [16] tzdb_0.4.0              InteractionSet_1.26.1    limma_3.54.2
## [19] Biostrings_2.66.0        annotate_1.76.0          systemPipeR_2.4.0
## [22] bdsMatrix_1.3-6         timechange_0.2.0        prettyunits_1.1.1
## [25] jpeg_0.1-10             colorspace_2.1-0        blob_1.2.4
## [28] rappdirs_0.3.3          apeglm_1.20.0           ggrepel_0.9.3
## [31] xfun_0.39               crayon_1.5.2            RCurl_1.98-1.12
## [34] graph_1.76.0            survival_3.5-3          glue_1.6.2
## [37] gtable_0.3.3            zlibbioc_1.44.0         XVector_0.38.0
## [40] DelayedArray_0.24.0     scales_1.2.1            futile.options_1.0.1
## [43] mvtnorm_1.2-2           DBI_1.1.3               Rcpp_1.0.11
## [46] xtable_1.8-4            progress_1.2.2          emdbook_1.3.13
## [49] bit_4.0.5              truncnorm_1.0-9         htmlwidgets_1.6.2
## [52] httr_1.4.6             gplots_3.1.3           RColorBrewer_1.1-3
## [55] pkgconfig_2.0.3         XML_3.99-0.14           farver_2.1.1
## [58] dbplyr_2.3.3           deldir_1.0-9           locfit_1.5-9.8
## [61] utf8_1.2.3             tidyselect_1.2.0        labeling_0.4.2
## [64] rlang_1.1.1            munsell_0.5.0          tools_4.2.3
## [67] cachem_1.0.8           cli_3.6.1              generics_0.1.3
## [70] RSQLite_2.3.1          evaluate_0.21           fastmap_1.1.1
## [73] yaml_2.3.7            knitr_1.43             bit64_4.0.5
## [76] caTools_1.18.2         AnnotationFilter_1.22.0 KEGGREST_1.38.0
## [79] RBGL_1.74.0            formatR_1.14            xml2_1.3.5
## [82] compiler_4.2.3         rstudioapi_0.15.0       filelock_1.0.2
## [85] curl_5.0.1            png_0.1-8              geneplotter_1.76.0
## [88] stringi_1.7.12         highr_0.10             futile.logger_1.4.3
## [91] GenomicFeatures_1.50.4 lattice_0.20-45         ProtGenerics_1.30.0
## [94] Matrix_1.5-3           multtest_2.54.0        vctrs_0.6.3
## [97] pillar_1.9.0           lifecycle_1.0.3        bitops_1.0-7

```

## [100] irlba_2.3.5.1	rtracklayer_1.58.0	R6_2.5.1
## [103] BiocIO_1.8.0	latticeExtra_0.6-30	hwriter_1.3.2.1
## [106] ShortRead_1.56.1	KernSmooth_2.23-20	codetools_0.2-19
## [109] lambda.r_1.2.4	MASS_7.3-58.2	gtools_3.9.4
## [112] DESeq2_1.38.3	rjson_0.2.21	withr_2.5.0
## [115] regioneR_1.30.0	GenomicAlignments_1.34.1	Rsamtools_2.14.0
## [118] GenomeInfoDbData_1.2.9	parallel_4.2.3	hms_1.1.3
## [121] VennDiagram_1.7.3	grid_4.2.3	coda_0.19-4
## [124] rmarkdown_2.23	GreyListChIP_1.30.0	ashr_2.2-54
## [127] mixsqp_0.3-48	bbmle_1.0.25	numDeriv_2016.8-1.1
## [130] interp_1.1-4	restfulr_0.0.15	